Issued November 23, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF SOILS—BULLETIN NO. 77.

MILTON WHITNEY, Chief.

ORGANIC COMPOUNDS AND FERTILIZER ACTION.

BY

OSWALD SCHREINER,

Scientist in Charge of Fertility Investigations,

AND

J. J. SKINNER,

Scientist in Fertility Investigations.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1911.

BUREAU OF SOILS.

MILTON WHITNEY, *Chief of Bureau.*
ALBERT G. RICE, *Chief Clerk.*

SCIENTIFIC STAFF.

FRANK K. CAMERON, in charge of Physical and Chemical Investigations.
C. F. MARBUT, in charge of Soil Survey.
OSWALD SCHREINER, in charge of Fertility Investigations.
W J MCGEE, in charge of Soil Water Investigations.

SCIENTISTS IN FERTILITY INVESTIGATIONS.

Edmund C. Shorey.
M. X. Sullivan.
B. E. Brown.
J. J. Skinner.
F. R. Reid.
D. J. McAdam.
E. C. Lathrop.
J. H. Beattie.
A. M. Jackson.
H. Winckelmann.

Issued November 23, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF SOILS—BULLETIN NO. 77.

MILTON WHITNEY, Chief.

ORGANIC COMPOUNDS AND FERTILIZER ACTION.

BY

OSWALD SCHREINER,

Scientist in Charge of Fertility Investigations,

AND

J. J. SKINNER,

Scientist in Fertility Investigations.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1911.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., December 5, 1910.

Sir: I have the honor to transmit the manuscript of a scientific paper entitled "Organic Compounds and Fertilizer Action," by Dr. Oswald Schreiner and Mr. J. J. Skinner, of this bureau. This paper is an important contribution to the study of the organic materials of soil and makes clear a number of soil and fertilizer actions in their relation to crop production. I recommend that this paper be published as Bulletin No. 77 of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

The action of fertilizers on soils is a much contested question, but the weight of evidence is against the assumption that their effect is due altogether to the increase of plant food as such. If so simple an explanation were the true one, nearly a century of investigation of this problem by scientists of all civilized nations would surely have produced greater unanimity of opinion than now exists in regard to fertilizer practice. Thoughtful investigators everywhere are finding that fertilizer salts are influencing many factors which contribute toward plant production besides the direct nutrient factor for the plant. It is this additional influence of fertilizers which makes them doubly effective when rightly used and inefficient when improperly used. To this influence of fertilizers on soil and biological conditions is due their capriciousness when applied on the theory of lacking plant food, and any study which throws further light upon the mooted question is of direct help toward reaching that view of soil fertility and soil fertilization which will eventually result in a more definite, more rational, and more remunerative fertilizer practice than in the past, and thus bring about the more extensive use of fertilizers in agriculture.

Organic compounds harmful to plant growth have been isolated from soils and their chemical and physiological properties reported in earlier publications from this laboratory. In the investigation presented in this article several toxic organic substances that may occur in soils, though not yet definitely determined as soil constituents, have been studied in relation to their influence on plant growth and absorption of salts, especially in regard to the relation existing between the harmful effect of such substances and the remedial action of the fertilizer salts. The broad scope of the investigation involved many experimental details and it was possible to carry out the work as here presented only by the earnest cooperation of a number of workers in this laboratory. Especial mention should be made of the work of Mr. J. H. Beattie and Mr. A. M. Jackson in the prosecution of this investigation.

Oswald Schreiner,
In Charge, Fertility Investigations.

CONTENTS.

ILLUSTRATIONS.

PLATES.

TEXT FIGURES.

ORGANIC COMPOUNDS AND FERTILIZER ACTION.

INTRODUCTION.

In the present bulletin some of the results obtained in experiments with toxic organic substances in restraining their harmful effects by fertilizer mixtures of different composition will be given. In former bulletins [1] the results obtained with dihydroxystearic acid, a crystalline organic compound isolated from a number of unproductive soils, was presented, the principal conclusions reached from these investigations being: (1) Dihydroxystearic acid hinders the growth of wheat plants when this is present in solution in pure distilled water; (2) the compound is also harmful in the presence of nutrient or fertilizer salts in all ratios of the fertilizer elements, P_2O_5, NH_3, and K_2O; (3) the compound is more harmful in those ratios of fertilizer elements not well suited for plant growth; (4) the harmful effect of the compound is least in those ratios of fertilizer elements best suited for plant growth; (5) the compound appears to be relatively much less harmful in the presence of fertilizers mainly nitrogenous than in the presence of fertilizers mainly phosphatic or potassic; (6) the harmful compound modifies greatly the removal of fertilizer elements from the solutions. The quantity of phosphate and potash removed was less in the presence of the compound, but the nitrate was not so influenced, and, on the whole, the amount removed was even greater. (7) The compound modifies both amount and ratio of the three fertilizer elements removed from solutions, the ratio being higher in nitrogen, which was also the most efficient fertilizer element in decreasing the harmful effect, as above mentioned. Whether the action is on the compound direct or on the plant, enabling it to withstand the harmful effect or enabling it to reduce the quantity of the compound, is not determined. (8) The harmful compound has the additional effect of darkening the root tips, stunting root development, causing enlarged root ends, which are often turned upward like fishhooks, and inhibiting strongly the oxidizing power of the roots; (9) those fertilizer combinations which tend to increase root oxidation are also the combinations which overcome the harmful effects to the greatest extent.

In the investigation culture solutions containing the three fertilizer ingredients, namely, P_2O_5, NH_3, and K_2O, as calcium acid phosphate,

[1] The Isolation of Harmful Organic Substances from Soils. By Oswald Schreiner and Edmund C. Shorey. Bul. 53, Bureau of Soils, U. S. Dept. Agr. Some Effects of a Harmful Organic Soil Constituent. By Oswald Schreiner and J. J. Skinner. Bul. 70, Bureau of Soils, U. S. Dept. Agr.

sodium nitrate, and potassium sulphate, respectively, in all possible ratios of one, two, and three constituents, varying the individual ingredients in stages of 10 per cent were used, the concentration being 80 parts per million of $P_2O_5+NH_3+K_2O$ in each case. To a similar set of cultures there had been added 50 parts per million of dihydroxystearic acid to each culture. Wheat plants were then grown in these various cultures with and without dihydroxystearic acid, and observations were made in regard to general development, the effect on root growth and appearance, and on root oxidation, and at the termination of the experiment the green weight of the plant was taken. The solutions were changed every three days and an analysis was made, the phosphate, nitrate, and potash being determined, thus giving the concentration of these elements and their ratios existing at the end of every three-day period for comparison with the original concentration and ratio. This changing of the solutions was kept up for 24 days, thus making eight changes. In this work the methods described in Bulletin 31 for the determination of small amounts of such constituents were used. In the discussion and presentation of the results the triangular diagram as used in physical chemistry was employed, and has proven very useful as a guide in the work for the systematic handling of the experimental details. The results can best be presented or interpreted by its means.

The results obtained with this organic soil constituent, giving its effect on growth and absorption of plant nutrients from the various culture solutions containing such a wide range of fertilizer composition, showed the desirability of obtaining further information concerning the behavior of other organic bodies known to be harmful to plants. Though not actually isolated from soil, those studied are common constituents of plant débris, or produced therefrom, and so become, at least temporarily, components of the soil. The effects of a large number of such compounds on plant growth were reported in a former bulletin.[1] Of these compounds, cumarin was selected for the continuation of these researches, because it was quite harmful even in minute amounts, a few parts per million of solution having a noticeable effect on plant growth, and because it was a common constituent of a number of plants, the remains of which get into the soil.

EFFECT OF CUMARIN AND FERTILIZER SALTS.

Cumarin ($C_9H_6O_2$) is a lactone of orthocumaric acid which may be regarded as oxy-cinnamic acid. Cumarin is a substance which has been found in a number of plants in different families of the plant kingdom. Among others, cumarin has been reported in the following plants: In grasses, viz, Anthoxanthum (Sweet Vernal), Hierochloë

---

[1] Certain Organic Constituents of Soil in Relation to Soil Fertility. By Oswald Schreiner and Howard S. Reed. Bul. 47, Bureau of Soils, U. S. Dept. Agr.

(holy grass), Milium, Cinna,[1] in various dicotyledons, viz, Herniaria, Ruta (common beet), *Prunus mahaleb*, Dipterix, Melilotus (sweet clover), Toluifera, *Alyxia stellata*,[2] the tubers of *Vitis sessiflora*,[3] *Galium triflorum* (bedstraw),[4] *Asperula odorata* (woodruff), *Ageratum mexicanum*,[5] and *Liatris odoratissima*[6].

Orthocumaric acid and o-hydrocumaric acid (Melilotic acid) are found in Melilotus, the sweet clover,[7] in addition to cumarin.

Para-hydrocumaric acid, although it has not been reported in plants, is of interest on account of its relation to tyrosine. Through the action of bacteria, the amid group in tyrosine is broken up and ammonia liberated, thus giving rise to p-hydrocumaric acid. It is also of interest to note that Gosio [8] has found that cumarin may be formed from carbohydrates by the action of certain mold fungi, e. g. *Aspergillus glaucus*, *A. varians*, *A. novus*, and *A. flavescens*.

Solutions of cumarin were shown to be toxic to the alga *Conferva minor* by Klebs,[9] when used in strong concentrations.

The earlier experiment referred to showed that cumarin is extremely poisonous to wheat plants. At the end of five days the plants in 250 parts per million and stronger were dying, the roots and tops having made practically no growth. The root tips were swollen and slightly discolored, although the roots themselves were quite turgid. When the experiment was discontinued at the end of eight days the plants in the solutions of 100 parts per million were dead, although they had made a slight growth at the beginning of the experiment. The roots were discolored for a distance of 3 to 6 mm. from the tip and their surface was very slimy, due to the death of the outer layers of cells, which were then beginning to peel off. The leaves of the affected plants were short and broad. This was characteristic of all experiments with cumarin. The cumarin appeared to injure the meristematic tissue of the stem in such a way that only the first leaves were unfolded, and in most cases the sheathing leaf base was more or less swollen by the abnormal growth of the inhibited leaves within it.

The results just cited were attained in solutions of the cumarin in distilled water. The present investigation concerns itself with the effect of cumarin in the presence of nutrient salts as well, the essential constituents of these being present to the extent of 80 parts per million, but varying as to composition. The number of

---

[1] Poulsen, Bot. Centr., **15**, 415 (1883).
[2] Proc. Amer. Phar. Assoc., **36**, 581 (1888).
[3] Peckholt, Zeit. Allg. österr. Apothek.-Ver., 1893, 829.
[4] v. Cotzhausen, Am. Jour. Phar., **48**, 405 (1876).
[5] Molisch and Zeisel, Ber. bot. Ges., **6**, 353 (1888).
[6] Vide Just's Bot. Jahresb., 1874, II, 947.
[7] Zwenger and Bodenbender, Ann. Chem., **126**, 257 (1863).
[8] Atti. R. Accad. Lincei, Classe sci. fis. mat. nat. (V) **15** (2) 59, (1906); abstr. Jour. Chem. Soc., **90**, 699 (1906).
[9] Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

culture solutions of the fertilizer salts used was 66, this being the number requisite to obtain every possible ratio of P_2O_5, NH_3, and K_2O, in 10 per cent stages. The system employed, as well as all details of preparation, was the same as described in the similar investigation with dihydroxystearic acid already mentioned.

The triangular diagram is used as a guide. In this diagram, fig. 1, the apices, Nos. 1, 56, and 66, are the cultures which contain only the single salts, calcium acid phosphate, sodium nitrate, or potassium sulphate, respectively; that is, contain 100 per cent of P_2O_5, NH_3, or K_2O, respectively. The line of cultures between 1

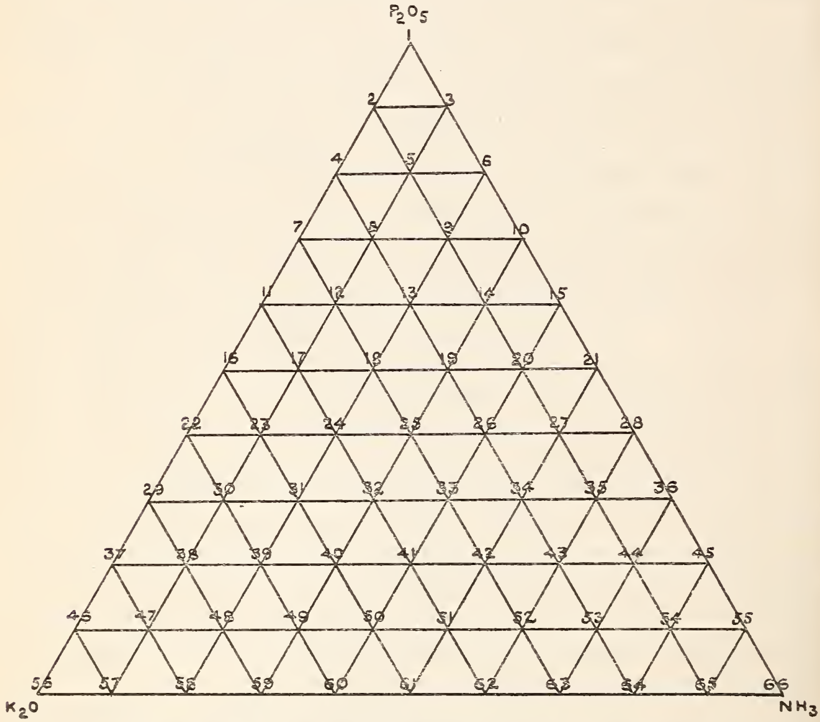

FIG. 1.—Triangular diagram, with the points representing the 66 culture solutions numbered.

and 66 contains mixtures of P_2O_5, and NH_3 in 10 per cent differences; the line of cultures between 1 and 56 contains mixtures of P_2O_5 and K_2O in 10 per cent differences; the line of cultures between 56 and 66 contains mixtures of K_2O and NH_3. The cultures in the interior of the triangle contain mixtures of all three constituents, differing in 10 per cent stages, one from the other, the composition depending upon its position in the triangle, those nearer the P_2O_5 apex consisting chiefly of phosphate fertilizer, those nearer the NH_3 apex chiefly of nitrate fertilizer, and those nearer to the K_2O apex chiefly of potash fertilizer. For a more detailed explanation

of the scheme and principles involved, the reader is referred to the earlier bulletin.

Two sets of these 66 culture solutions were prepared. One of them contained in every culture 10 parts per million of cumarin and a total concentration of 80 parts per million of the nutrient elements $P_2O_5 + NH_3 + K_2O$. The other set contained the nutrient salts only. The culture solutions were contained in wide-mouth bottles and 10 wheat seedlings grown in each culture after the manner described in Bulletin 70. The solutions were changed every three days, four such changes being made in each experiment, and were analyzed immediately after each change for nitrates, but the phosphate and potash were determined on a composite of the four changes. The green weight of the plants was determined at the termination of the experiment. As in the bulletin cited, comparisons are always made between solutions of like composition as far as the mineral salts are concerned. This comparison can, of course, be made between individual cultures of like composition or between groups of cultures when members of like composition occur in both groups. The first experiment with cumarin was set up on December 9 and discontinued December 21.

EFFECT OF CUMARIN ON GROWTH.

The effect of even so low a concentration as 10 parts per million of cumarin was strikingly noticeable in the difference between the plants growing in the two sets of cultures. The appearance of plants growing in solutions containing cumarin is very characteristic and totally different from the appearance produced by any other toxic compound worked with in this laboratory. The leaves are shorter and broader than is normal for wheat and only the first leaves are usually unfolded, the other leaves remaining wholly or partially within the swollen sheath. Such leaves as do break forth are usually distorted and curled or twisted. The appearance is shown only imperfectly in the figures of Plate I, but is so characteristic that the investigator can pick out the cumarin-affected plants from those affected by any other toxic body in the same experiment by a mere glance. This characteristic behavior of cumarin-affected plants becomes, therefore, also an indicator of the degree of its harmfulness in the cultures of different composition in this experiment. In addition to its effect on the tops, as just described, there was a general inhibition of root growth, as is the case with many other substances, notably the dihydroxystearic acid already described.

The effect of the cumarin was to depress the green weight of the plants from 100 to 88 in this experiment, although it was obvious from the appearance of the cultures that its effect was far from

uniform in all of the cultures and this is the most interesting feature of the experiment.

It will be recalled that with dihydroxystearic acid the more normal growth was observed in the nitrogen end of the triangle, but when the cumarin cultures were set out in this triangular form according to the composition of the culture solution, it became at once apparent that the result with the cumarin was not in harmony with the observation so repeatedly made with the dihydroxystearic acid. It was clear that the cumarin had an entirely different effect in the different culture solutions from that observed in the case of dihydroxystearic acid, which had responded most in the fertilizer combinations high in nitrate. With the cumarin this response was greatest in the fertilizer combinations high in phosphate. Attention should here be called to the fact that the different fertilizer combinations give different growths in the normal set and that the region of greatest growth lies in the ratios low in phosphate. The ratios high in phosphate give a lower growth than the ratios high in nitrate or potash, but the growth in all ratios is greater than that in pure distilled water. In the present experiment, for instance, the weight of control cultures grown in pure distilled water was 0.920 grams as compared with the culture in phosphate, which was 0.932, this being the lowest green weight in any culture of this series. For a detailed discussion of the phenomena associated with the interpretation of the normal growth of the cultures, Bulletin No. 70 should be consulted.

This influence of the phosphate on the harmful effect of the cumarin is perhaps most strikingly shown in the difference between the plants growing in the culture solution containing no phosphate whatever, namely, along the line 56 to 66 in figure 1, and the line of cultures immediately above this, containing 10 per cent phosphate in the fertilizer mixture. Where phosphate is entirely absent the effect of the cumarin is most marked. Above this line the harmful effect of the cumarin steadily decreases and in the upper part of the triangle disappears altogether so far as the eye can detect this in the appearance of the plants when comparing the normal and the cumarin sets.

Some of the cultures are shown in Plates I and II to illustrate this effect of phosphate on the cumarin cultures. In figure 1 of Plate I is shown the effect of cumarin (10 p. p. m.) in distilled water and figure 2 the effect of cumarin in the culture solution (No. 61) containing nitrate and potash only (40 p. p. m. NH_3 + 40 p. p. m. K_2O). It will be seen that although the two salts have very materially increased the growth in the control as compared with the control in distilled water, the effect of these two salts is very slight on the cumarin culture. As already mentioned, the result is quite different when

phosphate is also included in the culture. Figure 3 shows the plants growing in a solution containing 20 per cent phosphate in the fertilizer mixture (culture solution 43, containing 16 p. p. m. P_2O_5+48 p. p. m. NH_3+16 p. p. m. K_2O). That the marked improvement in the cumarin culture (2) is not due to the decreased amount of K_2O and increased amount of NH_3 over that in the culture represented in figure 2 is shown by the fact that the same improvement is shown in figure 1, Plate II, which represents culture 39, containing likewise 16 p. p. m. P_2O_5, but only 16 p. p. m. NH_3 and 48 p. p. m. K_2O. Figures 2 and 3 of Plate II represent the effect of still larger proportions of phosphate in the fertilizer mixture, showing that the growth in the cumarin cultures becomes more and more like the normal as the amount of phosphate increases.

The effect of the phosphate in overcoming the harmful action of the cumarin is shown in the green weight of the plants taken at the termination of the experiment. In Table I is given the green weight of the series of cultures containing the same amount of phosphate—that is, the series along any one of the horizontal lines in figure 1.

TABLE I.—*Showing the influence of phosphate in overcoming the harmful effect of cumarin.*

EXPERIMENT I.

P_2O_5 in fertilizer mixture.	P_2O_5 content in original solution.	Number of cultures included.	Total green weight of cultures.		
			Without cumarin.	With cumarin.	Relative (without cumarin=100).
Per cent.	*P. p. m.*		*Grams.*	*Grams.*	
0	0	11	21.773	15.370	70
10	8	10	22.408	18.835	84
20	16	9	20.339	17.140	84
30	24	8	17.143	15.350	90
40	32	7	15.008	14.085	94
50	40	6	11.188	11.150	100
60	48	5	9.113	9.005	99
70	56	4	6.915	6.485	94
80	64	3	4.171	4.330	104
90	72	2	2.388	2.530	106
100	80	1	.932	.955	102

The last column of the table gives the relative growth of the cumarin cultures. It will be seen that in those cultures in which no phosphate was present, the depression in growth caused by cumarin was greatest, being reduced to 70 per cent of the normal, and that the introduction of 8 parts per million of phosphate caused the growth to rise to 84 per cent of the normal. On further increasing the phosphate content to 16, 24, 32, and 40 parts per million the green weight rose to 84, 90, 94, and 100 per cent of the normal, respectively. From this point on the growth is practically as good in the cumarin set as in the normal control set, thus showing that, on the whole, the fertilizer combinations high in phosphate were practically able to overcome the harmful influence of the toxic cumarin.

The lessened toxicity of cumarin in solutions high in phosphate is also shown when the results of the experiment are grouped in such a way as to obtain all cultures containing 50 per cent and over of any one of the three constituents, P_2O_5, NH_3, and K_2O, as was done in the case of the dihydroxystearic acid experiment. This is accomplished by taking the cultures contained in the smaller triangles formed at each angle of the larger one, shown in figure 1—that is, the cultures contained within the subtriangles 1, 16, 21; 21, 61, 66; and 16, 56, 61, respectively. The sum of the green weights in these respective subtriangles is shown in figure 2 for the normal and the

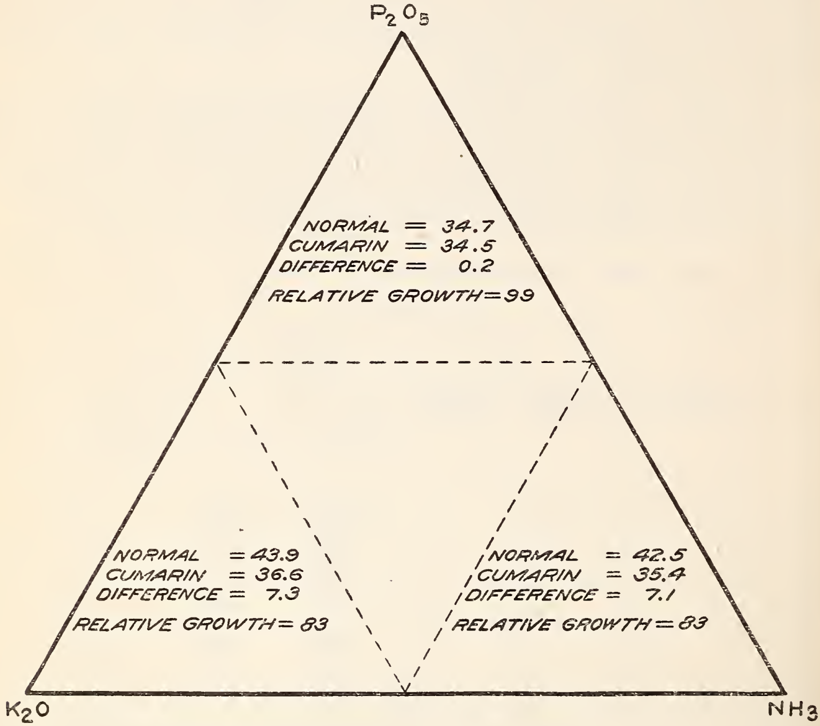

FIG. 2.—Showing the effect of the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers on cumarin.

cumarin set, together with the relative growth. The phosphate end shows that the growth in the cumarin set was nearly normal, 99 per cent, whereas the potash and the nitrogen end showed a growth only 83 per cent of the normal.

While this effect of the cultures high in phosphate was so striking, both in appearance of the cultures during growth and in the green weight obtained at the termination of the experiment, it was nevertheless so markedly different from the results obtained with the dihydroxystearic acid in this respect that the experiment was

repeated in order to obtain further information on this interesting and important observation. This set of experiments was carried on from January 12 to January 24 and was in all respects conducted as in the first experiment.

The cumarin-affected plants showed the same characteristic stunting of the leaves as in the former experiment and, moreover, again showed strikingly the influence of the phosphate in overcoming this effect, the general appearance of the entire triangle of cultures being similar to that already described. The effect of the cumarin was to depress the green weight from 100 to 75 in this second experiment, this being the average depression for all the cultures in the set. In Table II are given the green weights obtained in the mainly phosphatic, mainly nitrogenous, and mainly potassic solutions, obtained as already explained in figure 2.

TABLE II.—*Showing the effect of the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers on cumarin.*

EXPERIMENT II.

Fertilizers composed of 50–100 per cent of—	Green weight.		Decrease in green weight caused by cumarin.	Relative (without cumarin = 100).
	Without cumarin.	With cumarin.		
	Grams.	*Grams.*	*Grams.*	
Phosphate	35.5	30.2	5.3	85
Nitrate	42.3	31.1	11.2	74
Potash	42.4	29.5	13.9	70

Here, as in experiment 1, the toxicity of the cumarin was lessened most in the solutions high in phosphate, being 85 per cent normal, as compared with 74 and 70 per cent in the cultures high in nitrate or potash.

The line of cultures containing no phosphate whatsoever again showed the greatest effect of the cumarin; this harmful influence becoming less and less until complete recovery of the plants is noticed in the cultures containing higher amounts of phosphate. In Table III are given the green weights obtained in the cultures containing the different amounts of phosphate.

TABLE III.—*Showing the influence of phosphate in overcoming the harmful effect of cumarin.*

EXPERIMENT II.

P_2O_5 in fertilizer mixtures.	P_2O_5 content in original solutions.	Number of cultures included.	Total green weight of cultures.		
			Without cumarin.	With cumarin.	Relative (without cumarin = 100).
Per cent.	*Parts per million.*		*Grams.*	*Grams.*	
0	0	11	21.141	13.298	63
10	8	10	22.828	15.948	70
20	16	9	19.037	14.457	76
30	24	8	17.216	12.249	71
40	32	7	13.269	11.578	87
50	40	6	12.400	8.964	72
60	48	5	8.740	7.951	91
70	56	4	6.796	5.943	88
80	64	3	4.263	3.827	90
90	72	2	2.391	2.639	110
100	80	1	.873	.900	103

The total absence of phosphate shows a depressed growth equal to 63 per cent of the normal; this rises to 70 per cent on the addition of 8 parts per million and to 76 per cent on the addition of 16 parts per million, and so on upward, somewhat irregularly but definitely, until in the higher concentration of phosphate the effect of the cumarin is lost entirely.

A third experiment on this interesting effect of phosphate in overcoming the cumarin was made, but instead of culture solutions, soil treated with the various fertilizer combinations was used. The paraffined wire baskets were used, as described in earlier publications, two such cultures being used for each treatment. One set was treated with the 66 fertilizer combinations only, and the other set contained in addition to the fertilizer salts 50 parts per million of cumarin. The fertilizer added contained in each case 100 parts per million of $P_2O_5 + NH_3 + K_2O$. This soil set grew from February 8 to March 1.

The plants in the soil showed the same characteristic effect of the cumarin as in the other experiments and the effect was especially prominent in the series or line of cultures containing no phosphate. The growth in this series or line was depressed to 79 per cent of the normal and the addition of the 10 parts per million P_2O_5 raised this to 92 and the addition of 20 parts per million to 101. Above this the result was on the whole equal to normal except in the very highest phosphate cultures where the figures dropped to 89 and 85 per cent, respectively. The green weight in the cultures mainly phosphatic was 97 per cent of the normal, as compared with 94 and 93 per cent in the cultures with the mainly nitrogenous and potassic fertilizers.

FIG. 1.—WHEAT PLANTS GROWN IN DISTILLED WATER: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

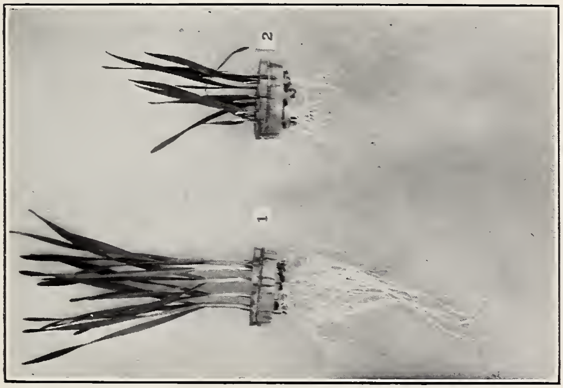

FIG. 2.—WHEAT PLANTS GROWN IN CULTURE SOLUTIONS CONTAINING A FERTILIZER MIXTURE COMPOSED OF PHOSPHATE 0 PER CENT, NITROGEN 50 PER CENT, POTASH 50 PER CENT: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

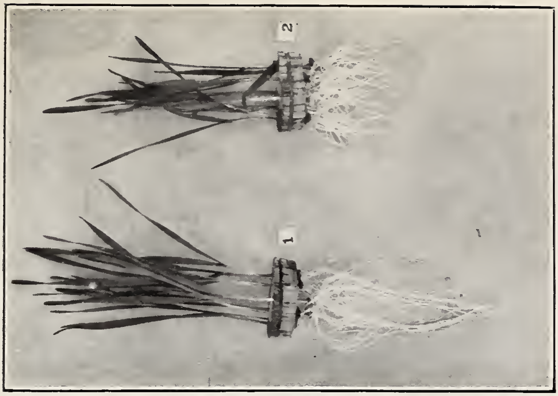

FIG. 3.—WHEAT PLANTS GROWN IN CULTURE SOLUTIONS COMPOSED OF PHOSPHATE 20 PER CENT, NITROGEN 60 PER CENT, POTASH 20 PER CENT: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

FIG. 1.—WHEAT PLANTS GROWN IN CULTURE SOLUTIONS COMPOSED OF PHOSPHATE 20 PER CENT, NITROGEN 20 PER CENT, POTASH 60 PER CENT: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

FIG. 2.—WHEAT PLANTS GROWN IN CULTURE SOLUTIONS COMPOSED OF PHOSPHATE 40 PER CENT, NITROGEN 30 PER CENT, POTASH 30 PER CENT: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

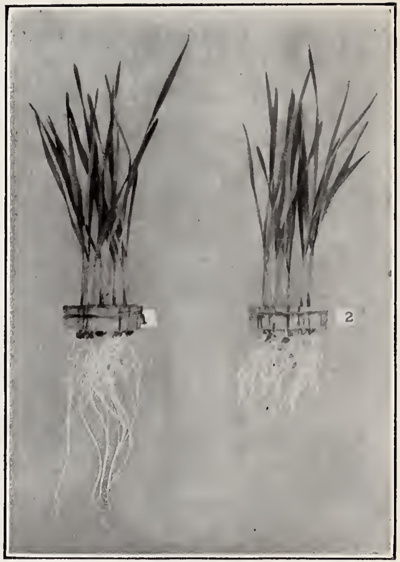

FIG. 3.—WHEAT PLANTS GROWN IN CULTURE SOLUTIONS COMPOSED OF PHOSPHATE 60 PER CENT, NITROGEN 20 PER CENT, POTASH 20 PER CENT: 1, WITHOUT CUMARIN; 2, WITH CUMARIN.

EFFECT OF CUMARIN ON CONCENTRATION.

The foregoing experiments show clearly the influence of cumarin on growth and the effect of phosphate in counteracting the harmful influence of the cumarin. There remains to be considered the influence of the cumarin on the concentration of the solution during the growth of the plant.

Mention has already been made of the fact that the concentration differences produced by the growth of the plants in the various cultures was determined by making an analysis for nitrate at the termination of every three-day change, and of the phosphate and potassium on a composite of the solutions from the four changes. It is thus possible to compare the results obtained under the so-called normal conditions without the cumarin and under the conditions where 10 parts per million of cumarin were present in the solutions. The 36 cultures comprising the fertilizer combinations in which all three fertilizer elements are present were consistently analyzed and only these are considered here.

The amount of total $P_2O_5+NH_3+K_2O$ removed from solution by the growing plants in the total number of 36 cultures was 1,379 milligrams under the normal conditions and 1,272 milligrams in the cumarin set. In Table IV are given the results for the P_2O_5, NH_3, and K_2O, separately, under the normal conditions and in the cumarin set of the first experiment.

TABLE IV.—*Total milligrams of P_2O_5, NH_3, and K_2O removed from the 36 culture solutions containing all three of these ingredients.*

EXPERIMENT I.

Ingredient.	Total absorption.		Relative (without cumarin =100).	Cumarin cultures above normal.
	Normal.	Cumarin.		
	Milligrams.	*Milligrams.*		*Per cent.*
P_2O_5	278.5	264.5	95	57
NH_3	482.6	415.3	86	22
K_2O	618.2	592.6	96	39

An examination of these figures at once discloses the fact that while the cumarin has decreased the absorption of these nutrient elements, it has not decreased it anywhere near the extent shown by dihydroxystearic acid in the experiment cited. The third column of figures gives the relative effect of cumarin on the absorption of each nutrient element and indicates that the phosphate and potash absorptions were the more nearly normal of the three, especially the phosphate absorption if the figures in the last column are taken into account. This column gives the percentage of the individual

cumarin cultures which showed an absorption equal to or greater than the corresponding culture without cumarin.

In the second experiment this effect is clearly marked, the phosphate absorption being 91 per cent of the normal, as compared with 78 and 87 for the nitrate and potash, respectively, which figures are again strongly supported by those in the last column of Table V. In this experiment the total absorption of $P_2O_5+NH_3+K_2O$ was 1,267 milligrams under normal conditions and 1,077 milligrams with cumarin.

TABLE V.—*Total milligrams of P_2O_5, NH_3, and K_2O removed from the 36 culture solutions containing all three of these ingredients.*

EXPERIMENT II.

Ingredient.	Total absorption.		Relative (without cumarin=100).	Cumarin cultures above normal.
	Normal.	Cumarin.		
	Milligrams.	*Milligrams.*		*Per cent.*
P_2O_5	285.7	260.3	91	44
NH_3	414.4	321.3	78	0
K_2O	567.3	495.8	87	22

While these figures indicate a somewhat more normal phosphate absorption in the cumarin set than normal nitrate or normal potash absorption, the figures are, nevertheless, not decisive enough to enable one to say definitely that the antagonism of the phosphate to cumarin, as shown in the growth of the plants, is due to this cause alone. A rigid examination of the complete data does not allow this conclusion to be drawn without at the same time suggesting the possibility of an external interaction between the lactone cumarin and the phosphate radical or possibly the acid calcium phosphate as a whole. The possible solution of this problem must be left for future investigation.

From the foregoing results it is apparent that the two toxic substances studied, dihydroxystearic acid and cumarin, show markedly different physiological properties, and are very differently influenced by fertilizer salts. Whether this is a direct action of the fertilizer on the organic body or through the medium of the plant cells, making the toxic substance and the particular fertilizer salt physiologically antagonistic, can not be definitely stated.

The cumarin so affected the normal development of the wheat as to cause stunting of leaf growth, with abnormal appearance associated with a slightly altered absorption of plant nutrients, both as to amount and ratio, the phosphate absorption being the more normal. The fertilizer combinations high in phosphate were the most effective in antagonizing the harmful effect of cumarin.

The dihydroxystearic acid also affected normal development, causing a decrease in top growth, but no abnormal appearance, the greatest abnormality being in this case observed in the root system, which was darkened and much stunted and showed swollen root tips, often bent into fish hooks, associated with a much altered absorption of nutrient elements both as to amount and ratio, the phosphate and potassium absorption being greatly depressed, the nitrate removal or disappearance being about as under normal conditions, but relatively much greater. The fertilizer combinations high in nitrate were the most effective in overcoming the harmful effect of this soil constituent.

In view of this widely different behavior of these two toxic substances, entailing the interesting observation that they responded differently to the different fertilizer combinations, it was thought desirable to obtain some results with other toxic substances. In the first place it would be interesting to see whether the result observed with dihydroxystearic acid, namely, response to the nitrate, was shown by another toxic body and thus throw a little more light on this phase of the question. For this comparison the aldehyde vanillin was selected. This was known to be toxic from former experiments, was known to be oxidized by the plant roots, and was further known to be more readily oxidized when nitrates were present,[1] and so should be a body which would behave much like dihydroxystearic acid.

EFFECT OF VANILLIN AND FERTILIZER SALTS.

Vanillin ($C_8H_8O_3$), the aromatic principle of the vanilla bean, is methyl protocatechuic aldehyde. It is probably not found as such in living plants, but exists in the form of a glucosid, which breaks down into vanillin and a sugar when the plant organs are dried. The glucosid, which gives rise to vanillin, probably occurs in a large number of plants. Vanillin, or substances which give rise to it, has been reported in oats (seeds and roots),[2] seeds of the white lupine,[3] asparagus shoots,[4] in raw beet sugar,[5] in Ilex leaves,[6] in dahlia roots,[7] etc. According to von Lippmann, vanillin seems to arise during the decay of wood under certain conditions.[8]

While vanillin has not been definitely isolated or identified in soils, much information has been obtained in the work of this laboratory to indicate its presence in some soils. Extracts reminding strongly

---

[1] Schreiner and Reed, Am. Chem. Soc., **30**, 85 (1908).
[2] de Rawton, Compt. rend., **125**, 797 (1897).
[3] Campani and Grimaldi, Chem. Centr., 1888, I, 377.
[4] von Lippmann, Ber. chem. Ges., **18**, 3335 (1885).
[5] Scheibler, Ber. chem. Ges., **13**, 335 (1880); Lippmann, ibid., 662.
[6] Polenske and Busse, Arb. kais. Gesundheitsamt, **15**, 171 (1899).
[7] Payen, Ann. chim., **24**, 209 (1823); von Lippmann, Ber. chem. Ges., **39**, 4147 (1906).
[8] von Lippmann, Ber. chem. Ges., **37**, 4521 (1904).

of vanillin have been obtained repeatedly and this property is carried over by shaking with a saturated solution of sodium bisulphite, acidulating, and shaking out with ether. In one case a minute amount of crystals having the appearance of vanillin was obtained, but the quantity was insufficient to effect identification.

Vanillin solutions were shown by Klebs[1] to be fatal to the alga, *Conferva minor*.

The solutions of vanillin were distinctly toxic to wheat seedlings in the earlier experiments conducted in this laboratory, 500 parts per million being sufficient to kill wheat plants in nine days. In all the stronger concentrations the roots were quickly killed and became slimy. Before death ensued the roots were able to oxidize part of the vanillin to a purple insoluble dye which was deposited upon the surface of the roots. The toxic effects of the vanillin were less marked upon the tops of the wheat plants than upon their roots; nevertheless the green weight of the tops in all solutions was less than that of the tops of control plants grown in distilled water. The green weight of tops of plants grown in a vanillin solution so dilute as 1 part per million was 91 per cent of the controls and their transpiration was 72 per cent of the controls.

In the experiment with vanillin here recorded, the same number of cultures, 66, containing all the fertilizer combinations possible in 10 per cent stages, was used as in the experiment with the dihydroxystearic acid and cumarin. The concentration of vanillin used was 50 parts per million. The duration of the experiment was from March 7 to March 19. The solutions were changed every three days as in the cumarin experiment already described, but no analysis of the solutions were made in this case. The green weight was, however, recorded.

EFFECT OF VANILLIN ON GROWTH.

The effect of the vanillin was not so marked on the tops as on the roots, although in the regions of better growth this also was not very prominent. The general appearance of the plants resembles the effect produced by dihydroxystearic acid much more than the effect produced by cumarin under the same circumstances. The region of greatest growth appeared also, as in the case of dihydroxystearic acid, to be shifted toward the nitrogen end of the triangle.

In the presence of these fertilizer salts the plant growth was 84 per cent of the normal, as an average of all the cultures.

For the present purpose, however, the growth in the cultures respectively high in phosphate, nitrate, or potash is of paramount interest. This grouping of the results obtained on the green weights

[1] Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

at the termination of the experiment is shown in figure 3. The relative growth in the cultures having 50 per cent and more of phosphate was 85 per cent of the growth without the vanillin; for the cultures mainly nitrogenous it was 88, and for the cultures mainly potassic it was 82. It will be observed that the vanillin depressed the growth least in the cultures high in nitrate, a result in harmony with previous observations on the toxicity of vanillin and its behavior with nitrate and in harmony with the action of dihydroxystearic acid.

Both vanillin and dihydroxystearic acid have reducing properties, that is, are themselves readily oxidized; both have an inhibiting

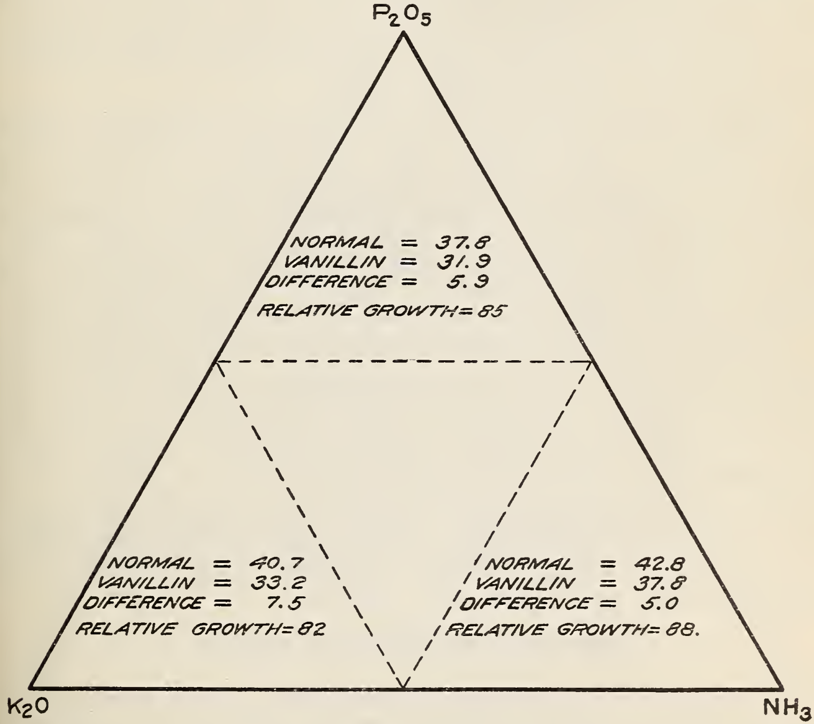

FIG. 3.—Showing the effect of the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers on vanillin.

effect on root oxidation and on root growth generally; both are overcome by the fertilizer combination which increases root oxidation to the greatest extent. It was consequently thought to be of interest to see what the effect of an organic compound having oxidizing properties would be on plants growing in these various fertilizer combinations. For this purpose quinone, shown to be toxic to wheat seedlings in a former research, was chosen, inasmuch as it is an oxidizing substance and therefore in strong contrast to the vanillin

with its decided reducing properties. This fundamental difference in the properties of the two compounds, it was thought, should show itself in an altered metabolism of the plants under the influence of two such widely different poisons and the scope of the present experiment as to different fertilizer combinations should lend itself to showing such differences in metabolism or fertilizer requirement and thus throw some light upon the behavior of crops in the field toward fertilizers under oxidizing conditions.

EFFECT OF QUINONE AND FERTILIZER SALTS.

Quinone, $C_6H_4O_2$, is a compound of interest in the study of soil problems, since Beijerinck [1] has found that a soil fungus, *Streptothrix chromogena*, has the ability to form quinone from proteids and since its formation in the soil from straw and other organic débris has been indicated by the researches of Emmerling.[2]

Quinone has been shown to be toxic to yeast by Laurent [3] and to the seedlings of various plants, algæ, and fungi by Furuta.[4] The last-named investigator found that in concentrations of 1,000 and 500 parts per million the roots of soy-bean and wheat seedlings were injured within a few hours and killed in three or four days. Filaments of algæ were killed in a concentration of 1,000 parts per million in three or four hours and fungi in a somewhat longer period.

In the earlier work mentioned [5] quinone was one of the most active poisons employed in the series of experiments. Wheat plants were killed in solutions containing 100 parts per million and seriously injured in solutions containing 50 parts per million. In the present experiments the concentration used was 10 parts per million. The fertilizer combinations and general technique were the same as in the preceding experiments with vanillin and cumarin. No analyses of the solutions were made in this experiment. The duration of the experiment was from March 23 to April 4.

EFFECT OF QUINONE ON GROWTH.

The effect of the quinone on the development of the wheat was in itself as definite, though perhaps not as characteristic, as the effect of cumarin. The effect of the latter substance was to produce short, broad, irregularly developed leaves and stunted tops; the effect of the quinone was to produce long, thin leaves, producing tall, slender plants so that at first glance the quinone in the concentration here used appeared to have had little effect on the growth of the plants. Closer inspection, however, shows the plants to be more slender and

---

[1] Centralbl. f. Bakt., Abt. 2, **6**, 2 (1900). Arch. Neerland. d. Sc. exactes et nat. (3), **3**, 327 (1900).
[2] Ber. Chem. Ges. **30**, 1869 (1897).
[3] Ann. Soc. belge. Micr., **14**, 29 (1890).
[4] Bul. Coll. Agr. Tokyo, **4**, 40' (1902).
[5] Bul. 47, Bureau of Soils, U. S. Dept. Agr. (1907).

weaker, although the leaves may be fully as long as the normal leaves. The effect of quinone on plant growth is, however, definitely shown by the decreased green weight. The root growth is also affected, as is the case with cumarin and other harmful organic substances.

The most interesting feature of difference between the normal and the quinone set of cultures, observable when both sets are arranged in triangular form according to the composition of the culture solution, is the apparent or real shifting of the greater growth toward the potash end of the triangle in the quinone set, accompanied by a

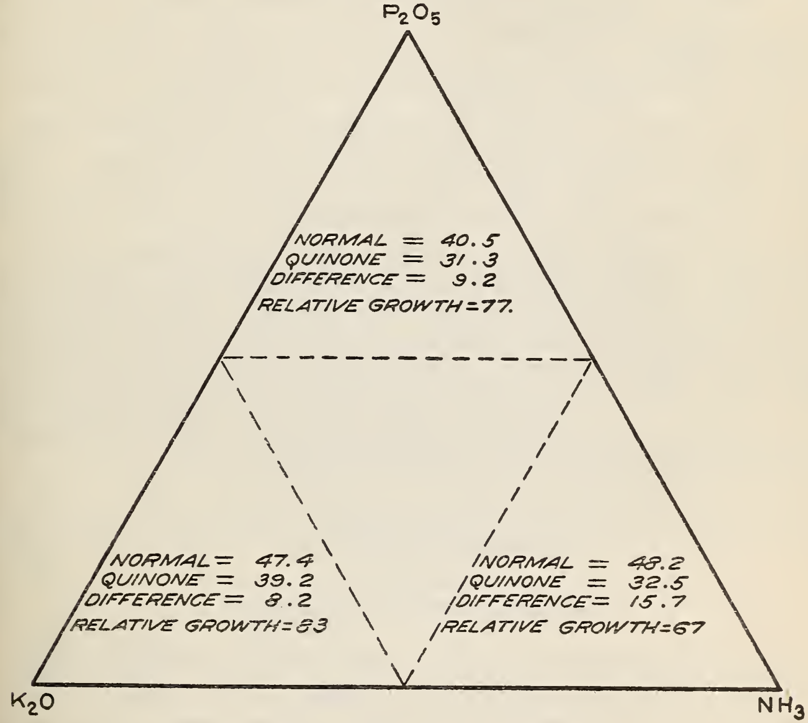

FIG. 4.—Showing the effect of the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers on quinone.

generally better relative growth in the potash angle. This observation would seem to show that the quinone effect was counteracted by the fertilizer combinations high in potash, whereas cumarin was undoubtedly affected by the phosphate fertilizers, as shown, and vanillin as well as dihydroxystearic acid, by the mainly nitrogenous fertilizers. This effect was not anticipated, but might easily have been, inasmuch as quinone is a strong oxidizing substance and potash salts are known from a previous research [1] to be retarders of root

[1] The Rôle of Oxidation in Soil Fertility. By Oswald Schreiner and Howard S. Reed. Bul. 56, Bureau of Soils, U. S. Dept. Agric. (1909).

oxidation, analogous to the opposite effect of vanillin, a reducing substance overcome by nitrate, known to stimulate root oxidation.

The green weights obtained at the end of the experiment bear out this observation. The relative growth in the quinone set was 75 per cent of the normal. The chief interest, however, centers in the comparative results obtained in the cultures containing 50 per cent and more of the phosphate, nitrate, and potash, respectively, in order to see which of these was the most efficient in antagonizing the action of quinone. The results of the grouping of cultures on this basis, made, as explained in the preceding experiment, is shown in figure 4. The mainly phosphatic fertilizer combinations showed a relative green weight of 77 per cent of the normal, the mainly nitrogenous 67 and the mainly potassic 83. It is observed that the potash fertilizers were the most efficient in overcoming the harmful effect of quinone.

This experiment with quinone was repeated and this time the solutions were analyzed as in the case of the cumarin experiment. This second quinone experiment lasted from April 8 to April 20. It showed the same general slender appearance of the plants, as well as again showing the influence of the potassium fertilizers, as above described. In this experiment the green weight in the quinone set as a whole was 79 per cent of that in the normal. The results for the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers is given in Table VI. The result with the phosphate fertilizers is 76, with the nitrate fertilizers it is 77, and with the potash fertilizers it is 85, again showing the greater relative efficiency of potash fertilizers in overcoming the deleterious effect of quinone.

Table VI.—*Showing the effect of the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers on quinone.*

EXPERIMENT II.

Fertilizers composed of 50 to 100 per cent of—	Green weight.		Decrease in green weight caused by quinone.	Relative growth without quinone
	Without quinone.	With quinone.		
	Grams.	*Grams.*	*Grams.*	*Per cent*
Phosphate	48.2	36.8	11.4	76
Nitrate	60.0	46.3	13.7	77
Potash	50.4	42.7	7.7	85

EFFECT OF QUINONE ON CONCENTRATION.

These quinone experiments show clearly the harmful influence of this substance on growth and the effect of potassium in restraining its action. In the second experiment the cultures were analyzed for phosphate, nitrate, and potassium, and it is interesting to inspect this data, as was done with the cumarin results. Only the 36 cultures having the combinations of all three fertilizer salts were considered.

The amount of total $P_2O_5+NH_3+K_2O$ removed from solution by the growing plant in the total number of 36 cultures was 1,568 milligrams in the normal set and 1,327 milligrams in the quinone set, showing a decrease in the sum total of P_2O_5, NH_3, and K_2O removed when quinone is present. In Table VII are given the results for the P_2O_5, NH_3, and K_2O, separately, under the normal conditions and in the quinone set.

TABLE VII.—*Total milligrams of P_2O_5, NH_3, and K_2O removed from the 36 culture solutions containing all three of these ingredients.*

EXPERIMENT II.

Ingredient.	Total absorption.		Relative (without quinone=100).	Quinone cultures above normal.
	Without quinone.	With quinone.		
	Milligrams.	*Milligrams.*		*Per cent.*
P_2O_5	300.4	173.6	58	8
NH_3	571.5	506.8	89	11
K_2O	696.5	646.5	93	36

An inspection of these figures indicates strongly that the potassium absorption in the presence of quinone has been more normal than that of the other two nutrient elements. This is shown both by the relative absorption in the third column and by the percentage of quinone cultures showing normal or greater absorption of P_2O_5, NH_3, and K_2O, respectively, as given in the last column.

We have, therefore, the interesting case of a toxic oxidizing body being overcome by a fertilizer salt having a restraining action on the normal oxidative power of the root, accompanied by a relatively greater absorption of this fertilizer element than under normal conditions.

DISCUSSION AND SUMMARY.

In the foregoing soil and water culture experiments with cumarin, vanillin, and quinone, the effects of these toxic substances on the development of wheat seedlings was demonstrable by three criteria:

First, by decreased green weight.

Second, by the morphological effects as shown by general appearance. Cumarin-affected plants have characteristic stunted tops, broad, distorted leaves; vanillin-affected plants are less characteristic, but show decreased growth of top and strongly inhibited root growth; quinone-affected plants are tall and slender, with thin, narrow leaves, in strong contrast to the cumarin-affected plants. The substances show, therefore, a markedly different behavior in detail, although all show a toxic effect in inhibiting growth.

Third, by decreased absorption of plant nutrients. The cumarin depressed potash and nitrate removal from nutrient solution more

than it did phosphate removal; the quinone, on the other hand, depressed phosphate and nitrate more than potash; the effect of vanillin was not determined in this regard. It might, however, be interesting to mention that dihydroxystearic acid, which appears to act much as vanillin did, depressed phosphate, and potash more than nitrate. In this respect again the influence of the various harmful substances was different.

The various fertilizer salts acted differently in overcoming the respective harmful effects of these toxic compounds. The mainly phosphatic fertilizers were the most efficient in overcoming the cumarin

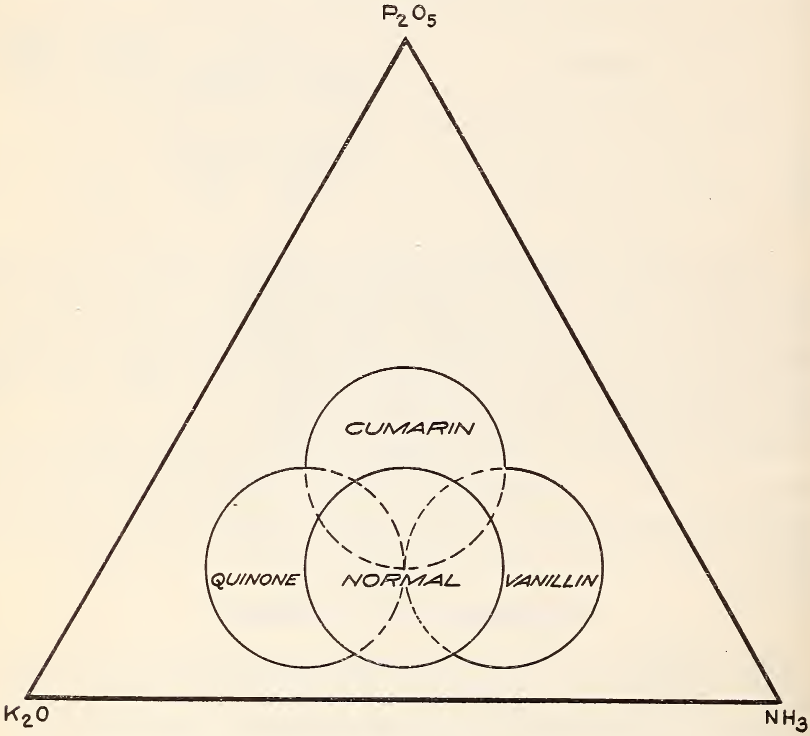

FIG. 5.—Showing region of greatest growth in normal, cumarin, vanillin, and quinone cultures.

effects; the mainly nitrogenous fertilizers in overcoming the vanillin effects; the mainly potassic in overcoming the quinone effects.

This different action of fertilizer salts on the toxic compounds is also illustrated in figure 5 by the diagrammatic representations of the regions of greatest growth obtained in the various experiments. The triangle represents the various cultures containing the fertilizer combinations, as is fully explained in figure 1 and the accompanying text.

Under normal conditions—that is, without any toxic body present—the greatest growth is found in those cultures low in phosphate

and about halfway between the nitrate and potash angles. This region of greatest growth is diagrammatically represented by the circle marked normal in figure 5. When cumarin was present in the cultures the effect was to shift the region of greatest growth in the direction of the phosphate angle, a condition which may be diagrammatically shown by the circle marked cumarin. With quinone, this region of growth was shifted toward the potash angle, and with vanillin, toward the nitrate angle, as illustrated in the diagram.

This shifting of the region of greatest growth was accompanied by a corresponding change in the absorption of plant nutrients, although this is not as marked as the green weight. All of these facts are in harmony with the conclusion drawn from the data already given—that phosphate fertilizers aided in overcoming cumarin; that potash fertilizers aided in overcoming quinone, and that nitrate fertilizers aided in overcoming vanillin and dihydroxystearic acid.

In regard to the exact mechanism of the chemical or physiological character of the interactions between these toxic substances and the fertilizer salts, nothing definite can be said. Attention should, however, be called in this connection to the fact that the reducing substances, vanillin and dihydroxystearic acid, are antagonized by those fertilizer combinations which promote oxidation, and that the oxidizing substance, quinone, is antagonized by the fertilizer combinations which retard oxidation, thus indicating that there is some correlation between these functions. A discussion of the interaction of cumarin and phosphate fertilizers would be mere speculation in the present state of knowledge.

Attention must also be called again to the fact that the observations here recorded for phosphate, nitrate, and potash, were obtained with the salts, calcium acid phosphate, sodium nitrate, and potassium sulphate, and that the observed results may, therefore, be caused by these substances as a whole, that is, as combinations, rather than individual elements. For deciding this question, further investigation is necessary, involving experiments with other salts and combinations.[1]

These actions of the different fertilizer combinations or different fertilizer requirements, as they may be styled, show a certain parallelism with field observations on soils and their fertilizer requirements

[1] Since this bulletin went to press several other experiments with cumarin and phosphate have been completed. The phosphates used were the three sodium salts, monosodium phosphate, which is acid n reaction; disodium phosphate, which is neutral in reaction; and trisodium phosphate, which is alkaline in reaction. These salts were used to answer the question whether the result obtained with calcium acid phosphate was caused by the salt as a whole rather than by the phosphate radical contained therein, or by other specific qualities of the salt or other constituent parts, namely, by its acid properties or the fact that calcium is present in the compound. Each of the three salts had the same effect in ameliorating the harmful effect of the cumarin as had the calcium acid phosphate. The conclusion seems warranted that the peculiar action of these phosphate salts in ameliorating the cumarin effects is due to the phosphate radical whether direct or indirect, and not to the presence of any particular base or the acid or alkaline reaction of the nutrient solution.

and one is tempted to ask to what extent may the different fertilizer requirements of different soils or of the same soil under different conditions, be influenced by the same cause. That harmful bodies occur in soils has been amply shown and that these are influenced directly or indirectly by fertilizer salts is also clear from this and other researches. That the constitution of the organic matter varies from soil to soil and in the same soil under different conditions of aeration, drainage, and cropping is likewise clear. The presence of compounds inimical to plant growth by virtue of a property resembling that of any of the above-mentioned substances might therefore cause a different fertilizer requirement, a requirement which might even change from time to time, according to the nature of the biochemical reactions producing the body or according to the nature of the plant remains in the soil; in other words, according to the rotation, with its necessary altered soil management, and the altered biochemical changes produced in the different plant remains.

TABLE VIII.—*Average concentration in parts per million of P_2O_5, NH_3, and K_2O after growth of wheat in the culture solutions in the experiments with cumarin and quinone.*

Culture.	Original concentration.			Cumarin Experiment I.						Cumarin Experiment II.						Quinone Experiment II.					
				Normal.			Cumarin.			Normal.			Cumarin.			Normal.			Quinone.		
	P_2O_5.	NH_3.	K_2O.	P_2O_5.	NH_3.	K_2O.	P_2O_5.	NH_3.	K_2O.	P_2O_5.	NH_3.	K_2O.	P_2O_5.	NH_3.	K_2O.	P_2O_5.	NH_3.	K_2O	P_2O_5.	NH_3.	K_2O.
5.....	64	8	8	42.6	0	2.7	55.8	0.4	2.7	47.7	0.3	2.5	51.9	0.5	4.4	54.3	0	5.0	49.8	0.3	1.9
8.....	56	8	16	38.5	0	1.5	47.8	0.4	2.8	47.4	0	3.7	48.8	0.4	4.0	46.7	0	3.4	46.7	0.3	4.0
9.....	56	16	8	38.5	2.5	2.4	50.9	3.7	1.7	46.7	2.5	1.7	46.0	4.8	1.4	41.5	1.0	2.3	45.9	2.7	2.5
12.....	48	8	24	36.2	0.3	2.4	33.7	0	6.1	34.2	0	3.4	33.7	0.6	5.2	37.4	0	4.0	43.9	0.9	6.8
13.....	48	16	16	36.2	2.8	2.4	36.1	3.6	3.7	34.2	2.8	2.9	36.7	5.1	2.0	37.8	0	2.0	42.6	1.8	2.6
14.....	48	24	8	34.3	10.3	1.5	37.4	9.9	1.5	34.8	11.8	3.2	40.5	14.5	2.4	35.5	7.7	3.0	42.6	8.8	2.2
17.....	40	8	32	29.9	0.4	10.1	27.1	0.3	10.1	27.2	0.3	8.7	26.6	1.0	15.6	33.2	0	6.9	33.9	0.7	10.6
18.....	40	16	24	29.9	1.1	2.9	27.8	4.8	6.4	29.6	1.4	3.4	26.8	6.0	7.1	29.3	0.4	3.2	33.2	2.3	6.8
19.....	40	24	16	29.9	9.3	3.0	27.8	9.2	4.8	28.1	11.9	1.9	27.8	14.8	5.7	32.5	5.7	2.5	36.4	9.9	2.0
20.....	40	32	8	32.3	17.0	3.9	29.1	16.0	2.4	28.5	21.3	3.2	27.8	21.7	3.4	28.7	14.3	5.3	36.9	16.3	5.6
23.....	32	8	40	23.1	0.3	15.9	20.6	0.4	15.4	23.2	0.5	19.2	23.9	0.8	21.3	22.6	0	10.9	26.7	0.4	10.9
24.....	32	16	32	24.9	2.0	4.8	20.6	3.1	8.0	24.6	2.0	8.0	24.9	6.4	14.7	21.4	0	3.3	26.2	3.0	6.8
25.....	32	24	24	23.9	7.8	4.8	22.1	10.5	6.1	20.6	8.1	6.8	23.9	12.5	8.7	24.2	5.9	5.4	24.1	10.3	8.0
26.....	32	32	16	27.1	18.8	3.1	22.9	17.9	4.2	23.9	18.7	2.0	23.4	19.5	5.0	23.9	12.1	5.3	24.1	14.7	2.3
27.....	32	40	8	26.0	26.1	3.6	22.9	27.1	4.0	24.9	29.9	6.0	23.9	32.2	3.4	23.0	22.1	5.3	23.7	24.8	2.6
30.....	24	8	48	17.9	0.5	22.8	17.6	0.5	23.8	17.3	1.0	20.9	17.9	1.5	28.1	17.1	0	13.3	18.6	0.6	19.9
31.....	24	16	40	17.9	1.5	12.3	15.8	3.6	14.8	13.6	5.0	13.7	14.9	5.6	19.2	17.1	0	9.2	18.6	3.4	13.3
32.....	24	24	32	19.5	9.0	12.8	16.0	9.7	12.3	15.5	10.6	9.1	16.6	14.1	14.7	15.7	4.6	6.4	19.6	8.3	10.6
33.....	24	32	24	17.3	14.0	3.6	17.3	19.1	6.8	17.3	17.0	4.4	16.0	22.7	10.1	15.5	12.6	6.0	19.6	15.6	5.6
34.....	24	40	16	18.7	24.4	3.6	16.0	27.7	3.4	19.3	29.5	2.7	16.6	32.2	4.6	16.6	20.1	3.0	18.4	24.0	3.0
35.....	24	48	8	19.5	34.4	3.6	16.3	37.5	2.7	19.4	39.1	5.0	16.9	40.9	2.5	15.3	29.7	4.3	18.2	33.9	2.6
38.....	16	8	56	10.4	0.6	29.8	9.9	0.5	24.0	12.0	0.4	22.0	10.4	1.1	31.9	10.5	0	19.9	12.2	0.5	18.2
39.....	16	16	48	10.4	2.0	24.0	11.5	4.8	26.6	11.2	2.1	14.5	11.3	7.8	30.0	8.1	0	15.9	13.7	1.9	14.1
40.....	16	24	40	9.9	8.7	17.5	10.8	10.1	16.7	13.6	11.9	18.2	10.4	13.9	22.3	8.6	4.3	12.0	13.6	7.9	15.0
41.....	16	32	32	10.7	14.9	11.9	11.2	20.8	15.9	11.3	18.6	8.7	12.1	23.4	14.4	8.6	14.2	6.2	12.7	13.8	12.0
42.....	16	40	24	8.6	25.4	13.6	10.4	26.8	6.7	11.6	26.8	4.8	11.8	31.6	9.6	8.6	20.9	8.0	13.6	23.8	12.0
43.....	16	48	16	8.6	33.2	5.1	10.4	36.0	6.8	12.5	37.2	6.4	11.0	38.4	4.0	8.4	27.6	3.9	13.1	30.5	5.3
44.....	16	56	8	9.0	40.9	3.6	10.5	42.7	2.8	13.4	46.9	3.8	10.9	47.0	3.0	9.0	37.4	3.4	13.3	37.2	6.0
47.....	8	8	64	5.7	0.4	25.2	3.4	1.0	31.9	3.8	0.3	34.2	4.0	1.7	46.5	3.1	0	28.3	6.0	0 9	18.2
48.....	8	16	56	5.0	1.8	17.7	3.6	3.2	29.8	3.0	2.0	26.6	4.6	6.5	36.5	2.1	0.4	19.9	6.5	1.6	18.2
49.....	8	24	48	2.8	7.5	24.0	3.0	6.9	20.0	3.1	9.2	25.1	4.9	15.2	32.9	1.9	5.2	15.0	5.2	7.5	24.0
50.....	8	32	40	2.8	14.0	11.9	3.0	12.8	12.8	3.7	18.3	15.6	4.0	20.6	21.3	2.0	11.5	10.1	5.2	13.4	19.9
51.....	8	40	32	3.1	22.8	12.3	4.4	29.8	14.8	3.6	26.3	10.6	4.4	30.7	29.4	1.9	18.2	6.4	6.0	22.9	13.7
52.....	8	48	24	3.7	29.4	5.0	3.8	38.9	6.7	4.1	35.7	5.9	4.7	38.5	10.6	2.3	32.9	5.4	5.3	29.5	10.6
53.....	8	56	16	4.0	41.7	3.7	3.7	46.1	4.8	4.4	42.4	3.4	4.3	46.5	3.8	1.9	37.2	3.4	5.0	36.9	3.7
54.....	8	64	8	3.7	51.6	4.8	4.3	56.9	3.4	5.0	53.8	2.5	4.4	58.0	2.4	2.2	42.9	5.7	5.3	45.9	2.0

TABLE IX.—*Green weight of cultures, in grams, in the experiments with cumarin, vanillin, and quinone.*

Culture.	Fertilizer composition.			Cumarin Experiment I, Dec. 9–21. Cumarin, 10 parts per million.		Cumarin Experiment II, Jan. 12–24. Cumarin, 10 parts per million.		Cumarin Experiment III, in Soil, Feb. 8–Mar. 1. Cumarin, 50 parts per million.		Vanillin Experiment Mar. 7–19. Vanillin, 50 parts per million.		Quinone Experiment I, Mar. 23–Apr. 3. Quinone, 10 parts per million.		Quinone Experiment II, Apr. 8–20. Quinone, 10 parts per million.	
	P_2O_5.	NH_3.	K_2O.	Normal.	Cumarin.	Normal.	Cumarin.	Normal.	Cumarin.	Normal.	Vanillin.	Normal.	Quinone.	Normal.	Quinone.
1	100	0	0	0.932	0.955	0.873	0.900	2.596	2.200	0.870	0.850	0.923	0.795	0.900	0.827
2	90	0	10	1.193	1.180	1.251	1.232	2.779	2.380	1.227	1.120	1.652	0.965	1.402	1.178
3	90	10	0	1.195	1.350	1.140	1.407	2.786	2.589	1.373	1.050	1.029	1.200	1.975	1.382
4	80	0	20	1.165	1.490	1.316	1.220	2.310	2.684	1.270	1.013	1.353	0.989	1.529	1.154
5	80	10	10	1.670	1.500	1.757	1.477	2.570	2.968	1.861	1.670	2.060	1.475	2.169	1.974
6	80	20	0	1.336	1.340	1.190	1.130	2.780	2.370	1.200	1.130	1.237	1.535	2.169	1.610
7	70	0	30	1.354	1.260	1.401	1.305	2.709	2.770	1.396	1.060	1.617	1.053	1.729	1.256
8	70	10	20	2.014	1.870	2.031	1.672	2.824	2.915	2.067	1.617	2.277	1.710	2.159	1.900
9	70	20	10	2.114	1.970	2.107	1.691	3.084	2.785	1.987	1.710	2.370	1.739	2.749	2.183
10	70	30	0	1.433	1.385	1.257	1.275	2.870	2.719	1.356	1.061	1.520	1.140	2.480	1.723
11	60	0	40	1.300	1.430	1.200	1.375	2.670	2.815	1.379	1.104	1.500	1.050	1.610	1.234
12	60	10	30	1.990	1.795	1.975	1.915	3.118	2.930	2.000	1.534	2.660	2.043	2.404	1.978
13	60	20	20	2.013	2.060	2.187	1.920	2.700	2.700	2.394	1.890	2.780	1.914	2.860	2.230
14	60	30	10	2.090	2.160	2.037	1.647	2.580	2.759	2.190	1.947	2.400	1.635	2.950	2.430
15	60	40	0	1.720	1.560	1.341	1.094	2.699	2.689	1.610	1.335	1.430	1.275	2.500	2.050
16	50	0	50	1.155	1.545	1.553	1.454	2.889	2.440	1.400	1.025	1.479	1.219	1.680	1.510
17	50	10	40	1.963	1.910	2.183	1.392	2.632	2.709	2.307	1.475	2.309	1.869	2.614	1.970
18	50	20	30	2.405	2.155	2.447	1.759	2.820	2.423	2.817	2.104	2.850	2.350	3.074	2.340
19	50	30	20	2.255	1.995	2.427	1.407	2.800	2.843	2.577	2.224	2.900	2.199	3.187	2.383
20	50	40	10	1.975	2.240	2.010	1.810	2.770	2.673	2.119	2.918	2.550	1.733	3.174	1.943
21	50	50	0	1.435	1.305	1.780	1.142	2.829	3.098	1.363	2.103	1.595	1.456	2.924	1.522
22	40	0	60	1.423	1.600	1.380	1.162	2.629	2.709	1.330	1.137	1.458	1.200	1.529	1.247
23	40	10	50	2.270	2.165	1.955	1.703	2.650	3.048	2.400	1.700	2.578	1.690	2.370	2.149
24	40	20	40	2.443	2.275	2.255	2.000	2.890	2.459	2.890	2.095	3.110	2.459	3.340	2.299
25	40	30	30	2.706	2.175	1.975	1.850	3.260	3.009	3.108	2.170	3.174	2.209	3.044	2.250
26	40	40	20	2.350	2.420	2.331	1.662	3.070	3.143	2.404	2.300	2.794	2.145	3.134	2.252
27	40	50	10	2.213	1.980	1.935	1.690	2.805	2.970	1.950	1.909	2.390	1.650	3.029	2.532
28	40	60	0	1.603	1.470	1.438	1.511	2.909	2.829	1.300	1.140	1.680	1.315	2.909	2.608
29	30	0	70	1.134	1.280	1.256	1.180	2.674	2.800	1.262	0.900	1.314	1.235	1.458	1.269
30	30	10	60	2.181	1.880	2.310	1.485	2.989	2.689	2.432	1.680	2.214	1.703	2.689	2.248
31	30	20	50	2.602	2.095	2.662	2.067	2.589	2.670	2.662	2.220	3.150	2.353	3.074	2.320
32	30	30	40	2.282	2.235	2.572	1.725	2.683	2.750	2.902	2.000	3.440	2.115	3.444	2.580
33	30	40	30	2.924	2.190	2.901	1.505	2.957	2.851	2.930	2.055	3.048	2.105	3.170	2.553
34	30	50	20	2.450	2.270	2.357	1.630	2.389	2.959	2.580	2.050	2.582	2.027	3.159	2.603
35	30	60	10	2.000	2.025	1.792	1.565	2.563	2.805	2.090	1.907	2.218	1.557	3.020	2.420
36	30	70	0	1.570	1.375	1.366	1.092	2.618	2.905	1.329	1.539	1.748	1.486	2.424	2.069
37	20	0	80	1.250	1.335	1.369	1.072	2.878	2.750	1.470	0.979	1.600	1.767	1.524	1.349
38	20	10	70	2.250	1.865	1.977	1.682	2.548	2.920	2.078	1.749	2.200	2.219	2.577	2.240
39	20	20	60	2.640	2.195	2.712	1.557	2.798	2.670	2.328	2.082	3.180	2.550	3.244	2.605

TABLE IX.—*Green weight of cultures, in grams, in the experiments with cumarin, vanillin, and quinone*—Continued.

40	20	30	50	2.603	2.130	2.292	1.825	2.784	2.713	2.285	2.150	3.170	2.213	3.400	2.535
41	20	40	40	2.481	1.755	2.492	1.825	2.559	2.913	2.579	2.561	3.490	2 560	3.060	2.390
42	20	50	30	2.484	2.040	2.490	1.775	2.679	2.980	2.740	2.301	3.320	2 309	3.230	2.580
43	20	60	20	2.374	2.210	2.205	1.802	3.329	2.830	2.403	2.130	2.987	1.499	3.375	2.339
44	20	70	10	2.395	2.110	2.010	1.527	2.899	2.900	2.008	1.839	2.287	1.379	2.850	2.170
45	20	80	0	1.862	1.500	1.490	1.392	2.870	3.020	1.638	1.319	1.905	1 100	2.750	1.845
46	10	0	90	1.210	1.160	1.435	1.172	2.960	2.706	1.363	0.994	1.315	2.059	1.496	1.275
47	10	10	80	2.300	1.950	2.085	1.680	2.940	2.800	1.823	1.726	2.109	2 050	2.386	2.029
48	10	20	70	2.470	1.920	2.741	1.495	2.830	2.970	1.679	2.206	2.879	2.280	2.737	2.345
49	10	30	60	2.725	2.290	2.715	1.625	2.658	2.775	2.289	2.126	3.419	2.060	3.093	2.685
50	10	40	50	2.403	2.275	2.397	1.845	3.068	2.488	2.685	2.267	3.318	2.581	3.533	2.439
51	10	50	40	2.783	1.740	2.559	1.857	2.738	2.609	2.970	2.610	3.749	2.270	3.723	2.800
52	10	60	30	2.850	1.995	2.515	1.592	3.050	2.519	2.480	2.120	3.390	1.970	2.955	2.400
53	10	70	20	2.050	2.095	2.620	1.682	2.798	2.360	2.397	2.227	2.998	1.931	3.250	2.430
54	10	80	10	1.935	1.840	2.155	1.550	2.450	2.650	2.107	2.032	2.278	1.300	3.215	2.353
55	10	90	0	1.682	1.570	1.606	1.450	3.070	2.420	1.740	1.420	1.810	0.921	2.784	2.008
56	0	0	100	1.305	1.205	1.270	0.537	2.720	2.150	1.190	0.990	1.200	1.574	1.210	1.239
57	0	10	90	1.693	1.025	1.733	1.213	2.931	2.248	1.685	1.205	1.843	1.850	2.030	2.030
58	0	20	80	2.005	1.150	1.842	1.490	2.965	2.377	2.030	1.360	2.220	1.759	2.607	2.193
59	0	30	70	2.201	1.445	2.302	1.174	2.900	2.467	2.190	1.522	2.370	1.650	2.680	2.319
60	0	40	60	2.340	1.510	2.008	1.027	3.023	2.469	1.970	1.709	2.490	1.750	2.453	2.325
61	0	50	50	2.255	1.375	2.364	1.067	3.078	2.210	2.120	1.458	1.920	1.470	2.603	2.365
62	0	60	40	2.363	1.485	2.218	1.267	3.015	2.454	2.252	1.390	2.006	1.550	2.543	2.025
63	0	70	30	2.280	1.285	2.017	1.430	3.179	2.378	2.022	1.708	2.122	1.371	2.190	1.945
64	0	80	20	1.872	1.840	2.097	1.485	3.175	2.550	1.809	1.743	2.098	1.420	2.414	1.925
65	0	90	10	2.034	1.710	1.663	1.288	2.850	2.640	2.013	1.676	1.720	1.250	2.589	1.868
66	0	100	0	1.425	1.340	1.627	1.320	2.809	2.020	1.507	1.205	1.370	1.230	2.079	1.450